APPENDIX K: 6% PAGE PLUS™ - OPTIONAL PROCEDURE

1. While working in a fume hood prepare a 6% PAGE Plus™ gel matrix solution as follows:

   1.1 Add 18 g of urea to a 50 mL conical tube.

   CAUTION: Urea dust particles should not be inhaled.

   1.2 Add 24 mL of Type 1 Water to the container.

   1.3 Add 7.5 mL of 40% PAGE Plus™ gel solution to the container.

   1.4 Add 5 mL of 10X TBE to the container.

   1.5 Swirl the 50 mL conical tube to mix the solution thoroughly, then heat the PAGE Plus™ gel solution briefly in a 50°C water bath to dissolve the urea particles.

   1.6 In a well ventilated area, add 300 µL of 10% ammonium persulfate and 30 µL of TEMED to the PAGE Plus™ gel solution.

   NOTE: 10% ammonium persulfate should be prepared the day of use.

   1.7 Using a 50 mL disposable pipette and an auto pipette, pipette the gel mix between the glass plates. Pipette the gel mixture slowly and maintain a continuous flow. Avoid creating bubbles. Carefully tap the plates to dislodge any air bubbles.

   1.8 Insert the 30 or 38 well comb at the top of the gel. Be careful not to form bubbles around the comb teeth.

   1.9 Allow the gel to polymerize in a horizontal position for at least 1 hour prior to setting up for electrophoresis.

   NOTE: The polymerized gel can be stored in the dark at room temperature by wrapping the gel in plastic wrap. Do not store the gel in the refrigerator once it has polymerized. To prevent the gel from drying, add a small amount of 1X TBE buffer or water to several paper towels and then lay the paper towel on the comb, and both sides and the bottom of the glass plates before wrapping with plastic wrap.

♦END